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TITLE: LIVE FIRE SUPPORT SERVICES

SUBTITLE: Expansion of Ngas Model to Include Nitrogen Dioxide Effects in the Rat

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**13. ABSTRACT (Maximum 200 words)**

The N-gas model is an empirical tool developed by the fire science community to predict toxic potency of various materials when burned based on the chemical nature of gases produced. This research is extending applicability of the N-gas equation to military scenarios by expanding the empirical formula to include the effects of exposure to NO2 (in rodents). Rats are exposed, nose-only and under controlled conditions, to nitrogen dioxide, singly and in combination with carbon monoxide, carbon dioxide and air. LC50 values are determined based on 30 minute exposure, followed by 2 week post exposure. Blood gas, hemoglobin and respiratory data are collected.

**14. SUBJECT TERMS**
Nitrogen dioxide, LC50, rodents, N-gas model, toxicity, acute inhalation effects, combustion gases

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( ) In conducting research utilizing recombinant DNA technology, the investigator adhered to current guidelines promulgated by the National Institutes of Health.

S M Smith

Date 1 June 94

S. M. Smith, JAYCOR
Principal Investigator

## TABLE OF CONTENTS

Live Fire Support Services:
Expansion of the N-Gas Model for Toxic Potency to Include $NO_2$ Effects
*MIDTERM REPORT*

**BACKGROUND**: Fire statistics in the United States reveal that the majority of persons who die in fires perish due to inhalation of toxic gases or combustion gases.[1] Similarly, for the soldier on the battlefield - the burning of fuel, propellants, insulation or other materials produces a wide variety of harmful gases. These gases can accumulate inside sealed armored fighting vehicles and remain at high levels for relatively long periods of time. Although hundreds of chemical species may potentially be present, only a few compounds are responsible for most of the toxicity. [2] These are generally the same toxic gases responsible for most fatalities in residential and aviation fires although concentrations and durations differ. [3] [4]

During the 1970's, knowledge about the toxicity of (burning) materials was considered a significant "missing link" in the evaluation and understanding of fire hazard. The need for a small-scale laboratory procedure to ascertain the toxic potency of the combustion products was highlighted by research [5] which indicated that the combustion products from an experimental fire-retarded rigid polyurethane foam caused seizures and death in rats, while the same foam without the fire-retardant did not produce any abnormal neurological effects. The toxicity of the foam was ultimately traced to the formation of a bicyclic phosphate ester in the smoke. This result raised an alarm about the possible presence of "supertoxicants" or unexpected compounds in smoke from burning or smoldering materials. Since the presence of this bicyclic phosphate ester

would not have been detected by ordinary chemical analysis of the smoke, this research also emphasized the need for animals as measurement "instruments". The need for a combined biological and chemical approach was obvious. The observation of adverse effects in rodents would indicate the presence of unusual toxicants or synergistic effects of combined toxicants which might not be discovered by routine chemical analysis. [6]

A number of organizations, including the National Bureau of Standards (now known as NIST, the National Institute of Standards & Technology) embarked on extensive research programs to develop standardized methods to assess and predict the toxic "potency" of various materials under controlled combustion conditions. [7]

Despite differences in approach, all of these methodologies had the following elements in common: laboratory animals were exposed under controlled conditions to smoke & combustion by-products atmospheres produced by the burning of various materials under prescribed conditions, including control of temperature, time of exposure, air temperature, availability of oxygen, combustion temperature, air movement and air flow. Most fire protection/prevention researchers used $LC_{50}$ as the endpoint although some work has been done using incapacitation levels. [8] The results were normally reported as the mass of the substance (under test) which was consumed to produce the effect.

Concurrent with developing an accepted standardized test methodolgy for assessing toxic potency of combustion by-products, researchers at NIST also

developed a mathematical model or formula to "predict" the toxic potency of mixtures of these gases. This model, known as the "N-Gas Model", was based on the premise that a small number ("N") of gases in smoke account for the majority of observed toxic potency. The lethality of each of these gases was determined for laboratory animals using pure gases rather than actual combustion atmospheres; similar measurements for combinations of these gases indicated whether the gases are additive, synergistic, or antagonistic. [9]

*NOTE:* Additive effects are defined as the case in which the predicted (and observed) response due to a combination of agents is equal to the sum of responses observed when both agents are administered separately. Toxicological interactions are considered to be synergistic when the response to combined agent exposure exceeds the sum of responses when each agent is administered separately. Antagonistic responses occur when the response to combined agent exposure is less than the sum of responses to individual agent exposures. Additionally, there are two distinct types of additive responses: "dose additive" in which two or more agents target the same receptor with similar efficiencies, but not necessarily the same potencies or "effect additive" in which agents act on different targets to produce similar effects. [10]

The results of these mixed gas studies were reduced to an algebraic equation which has been empirically determined for the exposure of rats to mixtures of $CO_2$ (carbon dioxide), CO (carbon monoxide), HCN (hydrogen cyanide), reduced $O_2$ (oxygen) and HCl (hydrogen chloride).

The concept that simple additivity may be sufficent to explain the toxicity of

mixtures of fire gases was originally proposed by Tsuchiya and Sumi [11] Significant work was also done in this area by Hartzell [12] who proposed the term "Fractional Effective Dose" or FED, for naming the variable which quantifies what fraction of a lethal dose the animal has received.

Huggett [13] suggested that the actual dose delivered to an animal via inhalation cannot normally be quantitatively determined and that we instead consider an exposure dose, defined as the product of the gas concentration in the atmosphere multipied by the time of exposure. This term is known as "Fractional effective Exposure Dose" or FED. For the case of simple additivity of effects, the FED is simply

$$= \sum \left( C_i \, dt \right) / LCt_{50}\,(i)$$

where Ci is the concentration of the ith gas species and LCt50 (i) is the lethal concentration * time product for the gas species. Experimental work conducted by NIST (and others) has borne out that mixtures of important toxic gases follow this relationship, with modification.

The most current form of the N-gas model as developed by NIST researchers is as follows [6].

$$FED = \frac{m[CO]}{[CO_2]-b} + \frac{[HCN]}{LC_{50}(HCN)} + \frac{21-[O_2]}{15.6} + \frac{[HCl]}{LC_{50}(HCl)} + \frac{[HBr]}{LC_{50}(HBr)}$$

Where

FED = A calculated number representing the concentrations & interactions of the various gases in the exposure atmosphere).

m,b = Empirical constants derived from experimental curves, and incorporating the "synergistic" effects of $CO_2$ concentration on the $LC_{50}$ of CO.[14]

[gas] = the concentration of a given gas in the exposure atmosphere given in ppm for all gases except $O_2$ which is expressed in volume %.

$LC_{50}$(gas) = concentration of a given gas lethal to 50% of the rats exposed for 30 minutes in a static environment, i.e. the $LC_{50}$ value. These concentrations are expressed in ppm for everything except $O_2$ which is expressed in volume %

The first term reflects the potentiation of CO by the presence of $CO_2$ [14]. Studies at NIST demonstrated that although $CO_2$ has a very low toxicity of itself (on rats), its effect on mixtures is not as slight as linear additivity would suggest. As the concentration of $CO_2$ increases, the (apparent) toxicity of CO increases; above 5%, the toxicity of CO begins to decrease again. The values

for m and b in the above equation represent the constants derived in a simple regression equation and are "m" = (-18) and "b" = 122000 for $CO_2$ concentrations of 5% or less and "m" = 23 and "b" = (-38600) when $CO_2$% exceeds 5%. Carbon dioxide also increases the toxicity of other gases currently included in the model as well as that of $NO_2$ [15] [16] However, for simplicity, the effect of the $CO_2$ was added into this equation only once. Since CO is generally the dominant toxicant in nearly all real fires, the $CO_2$ effect was merged into the CO factor (only).

The $LC_{50}$ values for 30-minute exposures for the other linear terms in the equation are as follows: HCN, 150 ppm [17]; HCl, 3800 ppm [18]; and HBr, 3000 ppm [19].

The third term in the equation arose because oxygen itself is not a toxicant, instead, its lack is what is toxic. Thus, the form for $O_2$ is $(21-O_2)$. The 30 minute $LC_{50}$ of $O_2$ is 5.4% which is subtracted from the normal concentration of $O_2$ in air, 21%.

Even with these non-linearities, the N-gas model equation still exhibited some systemic deviation from the ideal: 50% of the animals should die at an FED of 1.0; instead, the 50% lethality level corresponds to an FED of 1.1. Nonetheless, NIST researchers considered the model well enough established to be offered for engineering use, cautioning that nitrogen oxides, especially $NO_2$, needed consideration and that the antagonistic effects of $NO_2$ and HCN required further study.

**PURPOSE OF RESEARCH:** The research being conducted under the current 12-month support services effort is intended to meet the following objectives:

1. Assess the possiblities of applying the N-gas model approach to evaluating the "toxic potency" of various materials of military significance, considering that the existing model was developed to assess toxic potency of common commercial & residential construction and furnishings materials.

2. Analyze the *existing* N-gas model protocol for changes and enhancements which would better represent the situations existing in a military situation or combat fire scenario.

3. Utilizing unpublished data provided by researchers at NIST, revise the existing N-gas model to add $NO_2$.

4. Establish a small-animal exposure facility at the WRAIR Department of Respiratory Research laboratories, using equipment transferred from NIST researchers.

5. Validate the N-gas model as revised to include the effects of $NO_2$ using rodents.

6. Provide support as necessary to Department of Respiratory Research investigators in validation of the revised N-gas model in a large

animal protocol.

**PROGRESS/RESULTS:** Results to date for the stated objectives are addressed item by item in the following paragraphs.

OBJECTIVE # 1:

Assess the possiblities of applying the N-gas model approach to evaluating the "toxic potency" of various materials of military significance.

SUMMARY: The N-gas model procedure has been researched for applicability to the determination of toxic potency of materials of military significance. The procedure for animal exposures to combustion by-products is suitable to these determinations, however, there are accepted alternate procedures which do not utilize animals and are more appropriate for routine screening purposes.[20] The real strength of the N-gas model methodology is the potential to develop it into a mathematical model for the prediction of toxic effects from mixtures of combustion gases.

An extensive literature search was conducted to survey the current state of toxic potency testing. A compilation of various materials of military significance is being assembled for possible toxic potency evaluation testing.

The term "toxic potency testing" refers to the evaluation of a sample material or

composite to determine if hazardous levels of toxic gases will be released during combustion. Existing methodologies share the following constraints:

a) The conditions of burning are controlled, therefore combustion products produced may not be the same products or concentrations as those produced in a large-scale or "real" fire.

b) The test atmosphere is monitored for certain gases, typically carbon monoxide, hydrogen cyanide, nitrogen oxides and hydrogen halides. Other potentially toxic combustion by-products will go undetected.

c) The levels of gases measured are compared to an arbitrary standard for assessment of toxic hazard levels. These levels vary greatly and are often based on literature values rather than actual biological or animal model studies.

The N-gas model protocol in its current form has been validated against a large-scale combustion scenario for a few typical residential construction and furnishing materials. [21] As a result of this study, it was determined that the N-gas model combustion exposure protocol does not produce the same combustion atmosphere as the full-scale test *but*, given knowledge of the combustion atmosphere in an enclosure, the N-gas model formula *can provide* accurate predictions of the occurrence of animal deaths.

Since the N-gas model uses animals in the exposure phase, there is little chance that unknown toxicants will go undetected. Identification of the unknown

toxicants remains an involved & technological challenge, but identification is not a critical requirement of a screening procedure.

The commercial aviation community and the Federal Aviation Administration have developed a number of acceptable screening tests for toxicity. These include Airbus Industries procedure ATS1000.01, Boeing Aerospace procedure BSS 7239, British Aerospace BAEP 4623, and Douglas Aircraft DMS 2294. The hypothesis that the Boeing procedure will yield combustion products most closely duplicating the species of combustion products found in Live Fire tests will be further investigated.

The determination of accurate and reproducible $LC_{50}$ (or other selected endpoints) is one of the objectives of the research being conducted in this study.

OBJECTIVE # 2:

> Analyze the *existing* N-gas model protocol for changes and enhancements which would better represent the situations existing in a military situation or combat fire scenario.

SUMMARY: Literature review suggests that the N-gas model methodology is a good candidate methodology for application to military scenarios.

The N-gas model (as it currently exists) is based on thirty minute constant level exposures at normal room temperature and under static conditions (ie without significant airflow) After analyzing typical conditions reported during Live Fire

testing conducted by the Live Fire Directorate at Aberdeen Proving Grounds, the following changes and enhancements to the N-gas model exposure conditions will be explored. [NOTE: The protocol will be amended as needed to accomodate these conditions]

a) Shortening the exposure interval - thirty minutes is unrealistically long for conditions to remain static. Two, five and ten minute exposures are more representative of conditions encountered in an actual combat scenario.

b) Adding a term (into the equation) to account for temperature effects - thermal gradients in the Live Fire test environment can be significant and would certainly contribute to survivability, given the same concentrations of toxic gases. Not only does increasing temperature drive the kinetics of the chemical and biochemical interactions, it also causes detrimental effects of its own. The Federal Aviation Administration (FAA) has developed empirical formulas to gauge the effects of elevated temperature on incapacitance. [22] The incorporation of a modified thermal gradient factor will be investigated.

c) Increasing gas concentration levels - researchers at Aberdeen Proving Grounds have reported levels of NO2 exceeding 10,000 ppm. [23] Although such high concentrations disperse very quickly, refinement of the N-gas model and procedure to look at very high exposures coupled with shortened time intervals would

allow more accurate representation of actual field conditions.

OBJECTIVE # 3:

Revising the existing N-gas model to incorporate unpublished data to be provided by researchers at NIST.

SUMMARY: The data required to accomplish this revision has not yet been made available. Although other researchers have done limited studies on the toxic effects of nitrogen dioxide [24] there are no published data on $LC_{50}$ values for thirty minute high-level acute exposures. The protocol for this research effort includes animal studies to repeat NOx exposure studies in the event this data is not forthcoming. [Reference Appendix A for a copy of the protocol]

OBJECTIVE # 4:

Establishment of a small-animal exposure facility at WRAIR Department of Respiratory Research laboratories, using equipment transferred from NIST researchers.

SUMMARY: Several of the instruments needed to set-up the gas analysis and distribution systems were not included in transferred inventory. Other equipment items were not in operating condition. Rather than investing in

expensive and time-consuming repairs or refurbishments, other government equipment available within WRAIR is being utilized where appropriate.

The following critical milestones have been accomplished:

1) The dedicated fume hood has been installed and certified.
2) A new exposure chamber (modified to meet requirements in the protocol) has been designed and is presently under subcontract for fabrication.
3) Available gas analyzers needed to complete portions of the planned testing have been installed.
4) An elaborate multi-gas delivery/sampling/recovery systems has been designed and submitted for estimates.
5) Necessary safety and protective equipment has been ordered.
6) A new computerized data collection system is presently being installed and connected to the WRAIR LAN system.

OBJECTIVE # 5:

Validate the N-gas model as revised to include the effects of $NO_2$ using rodents.

SUMMARY: Protocol "Expansion of the N-Gas Model for Prediction of Toxic Potency of Combustion Gases to Include $NO_2$ Effects in the Rat" has been prepared by the Principal Investigator. The draft protocol was circulated for WRAIR LACUC review and approval of the protocol was granted 24 May

1994. The protocol has been assigned number M03-94. The protocol, included as Appendix A to this report, details planned experimental techniques and exposure conditions. The protocol was written to allow for additional animal exposures as needed to repeat/expand the $NO_2$ studies (as conducted by NIST researchers) in the event the necessary data is not made available (See Objective # 3).

The experimental methodology described in the protocol is based on the methodology utilized by NIST researchers in their pure gas and gas combination interaction studies. Basically, the rodents are placed in restrainers providing for nose-only exposure and exposed for thirty minutes as described in the protocol.

There are three sets of exposures planned: the first series to separate the $CO/CO_2$ effects and terms, the second for validation and verification of the $NO_2$ $LC_{50}$ values, and the third to validate the revised model in rats.

OBJECTIVE # 6:

> Provide support as necessary to Department of Respiratory Research investigators in validation of the revised N-gas model in a large animal protocol.

SUMMARY: The small animal exposure system is being designed and installed so that it can be easily adapted to support large animal testing in gas delivery, analysis, sampling and recovery. No large animal study is presently in work, therefore no support is needed at this time.

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# APPENDIX A

# WRAIR PROTOCOL # M03-94

PROTOCOL NUMBER: M03-94

TITLE: Expansion of "N-Gas Model" for Prediction of Toxic Potency of Combustion Gases to Include $NO_2$ Effects in the Rat

DIVISION: Medicine

DEPARTMENT: Department of Respiratory Research

INVESTIGATORS:

Contract Personnel:

Principal Investigator:
S. M. Smith, JAYCOR

DEPARTMENT CHIEF:

M. A. Mayorga, LTC, MC

DIVISION DIRECTOR:

R. C. Smallridge, COL, MC

CONSULTING VETERINARIAN:

P. Schultheiss, MAJ, VC

CONTRACTING OFFICE REPRESENTATIVE (COR):

A. Januszkiewicz, PhD

PROTOCOL NUMBER:_____

Received by LACUC/LDS_____

Approved/Sent for Revision_____

Received (2nd Time) by LACUC_____

Date Approved/Disapproved by LACUC_____

Previous Number_____

ACTIVE/COMPLETED/TERMINATED_____

PROTOCOL TITLE: Expansion of "N-Gas Model" for Prediction of Toxic Potency of Combustion Gases to Include $NO_2$ Effects in the Rat

SHORT TITLE: Refinement & Validation of "N-Gas Model" to Add $NO_2$ Effects

PRINCIPAL INVESTIGATOR: S. M. Smith (Contractor) PI PHONE: (301) 295-2755

DEPARTMENT Respiratory Research DIVISION Medicine APC: WICS STO YH

ANIMAL REQUIREMENTS:

| Species | Strain | Age | WT | Sex (M,F,E) | Total Number | Max no. housed |
|---|---|---|---|---|---|---|
| Rats | Sprague-Dawley | 8 - 10 wks Note 1 | 250-350 gr Note 1 | Male | 232 | 232 Note 2 |

Note 1 - Age/weight at test time, after 10 day quarantine period.

Note 2 - Only 32 animals (max) at a time require housing

SPECIAL HOUSING REQUIRED? (YES/NO) If yes, explain or cite section in protocol:

No special housing reqts. Code*_____

ARE ANIMAL REQUIREMENTS RESTRICTED TO A SINGLE VENDOR? NO

AGRICOLA: DTIC:

Date: 22/02/94, 23/02/94, 17/12/93 Date: 11/01/94, 21/12/93, 17/12/93

Search #: GOL39J, two other searchs, no #'s Search #: GOK02J, GOL18N, GOL35K

WORDS SEARCHED AGAINST: carbon dioxide, respiration effects, LC50, combustion gases, nitrogen dioxide, nitric oxide, Fischer rat, rats, sheep, computer modeling, Los Alamos research (on NOx), See also pg 3 of protocol

INDEX KEY WORDS (At least 5 words: e.g. species, strain, condition studied, experimental intervention, body system studied): Rats, Fischer, Sprague-Dawley, acute inhalation LC50, nitrogen dioxide, respiration, lethality

BIOHAZARD: Yes/No NO

BIOSAFETY LEVEL: (1) 2, 3, 4 BIOHAZARD/BIOSAFETY NAMES:_____

Code*_____ REFERENCE PAGE IN PROTOCOL:_____

USDA CATEGORY Number of Animals in Category

| | Year 1 | Year 2 | Year 3 |
|---|---|---|---|
| N-Minimal, transient, or no pain or distress | 24 | | |
| D-Pain or distress relieved by appropriate Measures | | | |
| P-Unrelieved Pain or Distress | 204 | | |

For P, ATTACH APPENDIX C IN PL 92-02 and CITE JUSTIFICATION PAGE IN PROTOCOL): pg. 15, App. C

ALTERNATIVES CONSIDERATIONS: Does the protocol have any provisions that would qualify it to be identified as one that refines, reduces, or replaces (3R's) the use of animals in relation to other protocols or procedures performed in the past?

YES REFERENCE PAGE IN PROTOCOL Ref pg.3, pg 9 & pg. 19

PROCEDURE CODES:* 03, 16, 20, 62, 63, Bleeding - arterial catheter, Death as endpoint

LOCATION CODE:* 10

* To be filled out by consulting veterinarian

MANAGEMENT DATA SECTION

SHORT TITLE: Refinement & Validation of "N-gas Model" to Add $NO_2$ Effects

TASK: YH_____ START DATE: March 94

END DATE: Sept 97

APC: WICS

USDA CODE: P

SPECIES/STRAIN/STOCK

| | |
|---|---|
| Species: | Rats |
| Strain: | Sprague-Dawley |
| Sex: | Male |
| Weight: | 250 - 350 grams |
| Total Number: | 232 |
| Purchase Cost: | $ 2088 (At current GSA cost of $9/animal) |
| Avg. Days Housed: | 30 days (includes 7 day quarantine period) |
| Cost/Day/Animal: | $ 0.18/day |
| Maintenance Costs: | $ 1253 |
| Total Cost: | $ 3341 |
| Special Requirements: | None |

ALTERNATIVES/SPECIES RATIONALE:

This research is being done for the specific purpose of refining and expanding an existing empirical mathematical formula used to minimize numbers of animals required to determine the toxicity of the combustion by-products of various materials. Data obtained in this study will be used to supplement data obtained in previous testing to minimize animal use in the development of a comprehensive computer model for the prediction of toxic combustion by-products effects on combat soldiers.

Researchers at the National Institute of Standards (U.S. Department of Commerce) used the Fischer 344 rat in the original studies leading to the development of the "N-gas model". The Sprague-Dawley rat was used in initial studies, but the sharp growth curve of

the strain caused logistics problems - often the animals outgrew the exposure porthole restrainers before exposure studies could be completed. This is not expected to be a significant problem in the proposed studies of this protocol. The Sprague-Dawley has been selected because it is the standard for acute toxicological studies and is an outbred rather than inbred strain (Baker, H. J., editor, 1980). If, however, initial studies indicate a statistically significant difference in $LC_{50}$ values between the Sprague-Dawley and the Fisher 344, a decision will be made regarding reverting to the Fischer 344 for the study.

DUPLICATION:

The following literature searches were conducted by the WRAIR Library; no research efforts were found which duplicate the work proposed in this protocol. Keywords/phrases searched included: carbon dioxide (effects) on respiration rates; irreversible effects on reduced oxygen (in humans); carboxyhemoglobin levels (for) lethality (or) incapacitation effects (in) humans (or) rats; $LC_{50}$ studies (on) $NO_2$, NO, CO, $CO_2$, HCN, HCl, HBr (in) rats (or) sheep; computer modeling (of) combustion gases (or) smoke effects (in) animals (or) humans, NOx toxicity (in) rats (conducted by) Los Alamos National Laboratories, acute toxic gases effects (in) animals (or) humans.

| | | |
|---|---|---|
| 22 February 1994 | No # | MEDLINE, TOXLINE, AGRICOLA |
| 25 February 1994 | No # | MEDLINE, TOXLINE |
| 23 February 1994 | No # | MEDLINE, TOXLINE, AGRICOLA |
| 11 January 1994 | GOJ59J | TOXICOL, PHARM, CHEMLIT |
| 11 January 1994 | GOK02J | DTIC |
| 21 December 1993 | GOL20I | DTIC |
| 21 December 1993 | GOL18N | CHEMLIT, TOXICOL |
| 17 December 1993 | GOL39J | AGRICOLA, TOXICOL |
| 17 December 1993 | GOL35K | DTIC |

TRAINING:

The PI has over ten years of experience in the development of experimental methods for the assessing the potential for the generation of toxic combustion by-products for a variety of military applications. In addition, she has successfully completed the "Rodent & Lagomorph DoD Laboratory Animal Workshop" and "Aseptic Techniques for Rodent Procedures Workshop" conducted by the Walter Reed Army Institute of Research, Department of Veterinary Medicine.

All technicians involved with this protocol will have completed the "Rodent & Lagomorph DoD Laboratory Animal Workshop" prior to assisting in the experiments.

HAZARDS:

Animals will be exposed in a "nose-only" position to various combinations of toxic gases representing typical combustion by-products for an exposure time of 30 minutes. The entire exposure facility is under a fume hood which is vented to the outside. At the end of the proposed exposure times, the chamber will be exhausted through appropriate gas scrubbers to be neutralized prior to venting to the outside. The volume of air in the chamber and the levels of gas concentrations are diluted essentially to zero concentration upon release to the outside airstream and no possible hazard exists. The animals are not hazardous after exposure and can be handled normally. Toxic gas monitoring will be conducted in the laboratory area to ensure that ambient levels do not exceed OSHA limits for exposure (Ref 13, Code of Federal Regulations).

ASSURANCE STATEMENT:

"As the principal investigator, I acknowledge responsibility for the conduct of these procedures with animals. I hereby certify that the information provided is correct and reflects the procedures to be used. I further promise to conduct this work with animals in accordance with the procedures approved by the WRAIR Laboratory Animal Care and Use Committee (LACUC) and institutional policy. I will submit a revised animal protocol and obtain LACUC approval prior to making significant changes in the procedures as approved by the LACUC."

S M Smith (23 Apr 94)

S. M. Smith (JAYCOR)

## TECHNICAL DISCUSSION -- SECTION II

### I. NEED

Researchers at The National Institute of Standards & Technology Fire Research Center (NIST) have developed an empirical mathematical formula for assessing the toxic potential [the combinations & concentrations of toxic gaseous combustion by-products which will produce 50% lethality in Fischer 344 rats] of various materials under controlled combustion conditions (Babrauskas, et al, 1991). This formula is called the "N-gas model" and currently includes carbon monoxide, carbon dioxide, hydrogen cyanide, reduced oxygen, hydrogen chloride and hydrogen bromide. When the project was cancelled in 1992, research was planned to add nitrogen dioxide ($NO_2$) to the model. The Army has a need to continue this research to expand the N-gas model to include the effects of $NO_2$ singly and in combination with other common combustion by-products.

Nitrogen dioxide and nitric oxide (collectively referred to as NOx) have military significance, especially at levels well above ambient norms. These gases are often generated as a result of thermal pulses occurring when high explosive antitank (HEAT) or kinetic energy (KE) rounds penetrate armored combat vehicles (ACV), (Ripple, et al, 1989). This thermal pulse is sufficient to ignite essentially any combustible material in the vehicle and to initiate the formation of NOx (as well as other toxic fire gases). Although the threat of crew space fires has been minimized through improved stowage techniques, compartmentalization of munitions and the use of automatic fire extinguishing systems (AFES), the reality is exposure to brief but high concentrations of toxic gases can and does occur in the modern battlefield scenario.

## II. BACKGROUND

The Walter Reed Army Institute of Research, Division of Medicine, Department of Respiratory Research has been involved in non-fragment injury, especially blast overpressure and toxic combustion gas exposure effects research for the past fifteen years. The research supports the Army Health Hazard program and the Live Fire Test program which address hazards associated with weapons use and crew survivability in battlefield scenarios, respectively. The blast overpressure research has led to the development of a sophisticated computer model for predicting injury levels as a result of weapons fire and/or blast exposure. No such model exists for the prediction of injury, lethality or incapacitation due to toxic combustion gas exposures.

Organizations concerned with flammability safety, including the FAA (Federal Aviation Administration) and NFPA (National Fire Protection Association) have conducted studies of a wide variety of burning materials in order to determine the chemical nature of the combustion by-products. (Landrock, 1983). In an effort to standardize the diversity of procedures (and results) being reported, the Fire Prevention and Control Act of 1974 chartered the National Bureau of Standards (now known as National Institute of Standards & Technology or NIST) to develop a standard toxic potency test method. Their research led to the development of a methodology to assess the toxic potency of mixtures of combustion gases, based on the physiological interactions of a small number of individual gas components. Concurrent with the actual test procedures, a bioanalytical tool, known as the "N-gas model" was also developed. (Levin, B. C., 1992)

The "N-gas model" is an empirical mathematical formula expressing the probability of lethality (at 50%) of Fischer 344 rats when exposed to mixtures consisting of the following major fire gases: carbon monoxide (CO), carbon dioxide ($CO_2$), lowered oxygen ($O_2$), hydrogen chloride (HCl), hydrogen bromide (HBr), and hydrogen cyanide (HCN). In addition to providing some insight into the cumulative lethality effects of combustion gas mixtures, the N-gas model is employed to minimize the numbers of animals sacrificed in

determining the toxic potency of burning or combusted materials.

The N-gas model was developed through hundreds of studies on rats exposed to various combinations of pure gases and gas mixtures in air. The first studies involved carbon monoxide and air. Then the effects of carbon dioxide were evaluated and added to the model. Next reduced oxygen effects were studied. Hydrogen cyanide and the hydrogen halides effects were also evaluated as individual gases in air. Development of the model to this point has required over ten years of research and animal exposures.

The current form of the N-gas model is (Levin, B. C., 1992)

$$\text{N-gas value} = \frac{m\,[CO]}{[CO_2] - b} + \frac{[HCN]}{LC_{50}(HCN)} + \frac{21 - [O_2]}{21 - LC_{50}(O_2)} + \frac{[HCl]}{LC_{50}(HCl)} + \frac{[HBr]}{LC_{50}(HBr)}$$

where

N-gas value = A calculated number representing the concentrations & interactions of the various gases in the exposure atmosphere)

m,b = Empirical constants derived from experimental curves, (Levin, 1987) and incorporating the "synergistic" effects of $CO_2$ concentration on the $LC_{50}$ of CO.

[gas] = concentration of a given gas in the exposure atmosphere given in ppm for all gases except $O_2$ which is expressed in volume %.

(gas) = concentration of a given gas lethal to 50% of the rats exposed for 30 minutes in a static environment, i.e. the $LC_{50}$ value. These concentrations are expressed in ppm for everything except $O_2$ which is expressed in volume %

In theory, 50% of the exposed animals should die when the N-gas Value equals 1. In studies done at NIST, however, the experimental N-gas value corresponding to 50% lethality was 1.1 (95% confidence level of +/- 0.2). Researchers theorized that, since concentration-response curves for animal lethalities from smoke are very steep, the calculated values are close to the predicted $LC_{50}$ value if any of the exposed animals die (Babrauskas, et al, 1991).

Researchers at NIST were carrying out experiments to determine $LC_{50}$ values (in the Fischer rat) for the combustion products of various construction and areospace materials. In NIST's studies, a small specimen of the material being evaluated for combustion toxicity potential was burned under carefully controlled conditions. Concentration measurements of the toxic gases generated by the material were made, averaged and plugged into the N-gas model formula. Based on one or two test runs, the N-gas value of a given mass of the test material was calculated. The calculated $LC_{50}$ for the test material was then validated with two exposures using rats. These exposures involved target gas concentrations approximately at the predicted or calculated $LC_{50}$ value. Six rats were exposed, head only, for each experiment. The total exposure time was 30 minutes with a minimum 14 day post-exposure observation period.

Recent changes in the primary focus of NIST to advanced manufacturing techniques has resulted in cancellation of funding (for NIST researchers) for N-gas model development and toxic potency measurements. NIST no longer approves or conducts any research involving animal testing.

Using the experiments described in this protocol, the Department of Respiratory Research plans to modify, expand and adapt the existing N-gas model methodology and procedure to the evaluation of toxic gas effects (models) for both Live Fire Test and Army Health Hazard Assessments. There are several reasons to start with the N-gas model in its present form but a primary consideration is that proper application of the technique will reduce the numbers of animals which would otherwise be necessary to develop an injury

prediction model for toxic combustion gas effects.

## III. HYPOTHESIS AND/OR OBJECTIVE

The objectives of this study are as follows:

1. Enhancements to existing NIST "N-gas" procedure. The current N-gas model experimental procedure will be modified to more accurately reflect toxic combustion gas exposure situations relevant to the Health Hazard Assessments and Live Fire Test programs. Experimental technique will be refined to increase reliability and reproducibility also.

2. Addition of $CO_2$ as a separate term. The current N-gas model integrates $CO_2$ effects into the CO term. Although that was appropriate to the types of situations which NIST was investigating, it is not as applicable for the Army's needs. Experiments will be conducted as required to separate the terms.

3. Addition of the $LC_{50}$ values for $NO_2$. Experiments will be conducted as necessary to collect the data needed to expand the modified N-gas model to include $LC_{50}$ effects (in rats) of $NO_2$ and/or NO alone, together, and in combination with $CO_2$ and CO.

4. Validation of the revised N-gas empirical formula. Experiments will be conducted as needed to demonstrate the accuracy of the revised and expanded N-gas model in predicting lethality effects in the rat.

## IV. MATERIALS & METHODS

### A. Experimental Design

1. Modification of existing N-gas experimental procedure. Modification of the existing experimental technique will not require the use of any animals. These modifications will

include the following:

A. Evaluations of changes to the method of gas delivery to the test chamber. Premixing, on-demand delivery and in-situ mixing of gases will be evaluated to determine which method gives the most consistent distributions of test gases in the chamber for the exposure period.

B. Evaluation of sampling collection points. The chamber will be charged with a proposed test gas mixture under steady state conditions and gas concentrations will be measured at several locations. This data will be used to determine sampling points during actual exposures to ensure reproducibility.

C. Evaluation of stability of test gas mixtures. The exposure chamber will be charged with gas mixtures typical of those to be used during the animal exposures. Samples will be taken at five minute intervals and analyzed for content to determine if the gas concentrations are stable over the duration of planned exposure times.

D. Evaluation of methods to prevent stratification in the test chamber atmosphere. If studies of gas stability and sampling collection sites indicate the need, measurements will be conducted to sample the exposure chamber atmosphere with and without mechanical mixing.

2. The addition of $CO_2$ as a separate term. In the current N-gas model, $CO_2$ effects are included as part of the CO term, i.e., all of the potentiating effects of $CO_2$ are applied to CO. Although $CO_2$ is a known respiratory stimulant with probable effects on the $LC_{50}$ values of other toxic gases, researchers at NIST determined that CO is, by far, the main toxicant in a fire situation (especially before "flash-over") and, therefore, they combined the two gases into a single term. It is necessary to separate the contributions of the two gases to the $LC_{50}$ values in order to expand the N-gas model.

Groups of eight rats will be exposed for a predetermined time to various concentrations of CO in air, $CO_2$ in air and CO & $CO_2$ in air. Two of the animals will be selected at random for blood sampling; the other six animals will be observed during exposure and post exposure; the time of death will be noted. Typically, acute $LD_{50}$ studies utilize five to six animals. Blood samples (from the two selected animals) will be collected to monitor levels of toxic effects. These procedures are detailed in the sections B, "Animals" and D, "Test Procedures." Table I contains a matrix of proposed concentrations for this phase of the study. Data collected from this phase of the study will be used to refine the gas combinations and concentrations listed in Table II for the addition of $NO_2$ effects.

3. Validation of previously determined $LC_{50}$ value for $NO_2$ and determination of $CO_2$ concentration effects on $NO_2$ $LC_{50}$ values. Groups of eight rats will be exposed for 30 minutes to various concentrations of $NO_2$. Two of the animals will be designated for blood sampling; the remaining six animals will be observed during exposure and post exposure (14 days), the occurrence & time of death will be noted. Blood samples will be collected to monitor levels of toxic effects. These procedures are detailed in the sections B, "Animals" and D, "Test Procedures." Table II contains a matrix of proposed concentrations for this phase of the study.

**TABLE I**

**Separation of CO and $CO_2$ Effects**

| RUN # | CO conc, ppm | $CO_2$ conc, % | Air, breathing | Number of rats | Objective |
|---|---|---|---|---|---|
| | | | | | |
| A-1 | none | none | balance | 8 | Control set |
| A-2 | 1000 ppm | none | balance | 8 | Validate literature LC50 value |
| A-3 | 2000 ppm | none | balance | 8 | " |
| A-4 | 3000 ppm | none | balance | 8 | " |
| A-5 | 4000 ppm | none | balance | 8 | " |
| A-6 | 5000 ppm | none | balance | 8 | " |
| | | | | | |
| A-7 | none | 5% | balance | 8 | Control set |
| A-8 | 1000 ppm | 5% | balance | 8 | CO2 effect on CO LC50 |
| A-9 | 2000 ppm | 5% | balance | 8 | " |
| A-10 | 3000 ppm | 5% | balance | 8 | " |
| A-11 | 4000 ppm | 5% | balance | 8 | " |
| A-12 | 5000 ppm | 5% | balance | 8 | " |
| | | | | | |
| A-13 | TBD (1) | TBD | balance | 8 | Validate revised model |
| A-14 | TBD | TBD | balance | 8 | " |

TBD => To Be Determined (based on results of runs A-2 thru A-12)
LC50 => Lethal Concentration (to 50% of animals exposed)

## TABLE II

### Expansion of N-gas Model to Add $NO_2$

| RUN # | $NO_2$ conc, ppm | $CO_2$ conc, % | Air, breathing | Number of rats | Objective |
|---|---|---|---|---|---|
| | | | | | |
| B-1 | none | none | balance | none | Control, Use data from A-1 |
| B-2 | 50 ppm | none | balance | 8 | Verify NOx LC50 value |
| B-3 | 100 ppm | none | balance | 8 | " |
| B-4 | 150 ppm | none | balance | 8 | " |
| B-5 | 200 ppm | none | balance | 8 | " |
| B-6 | 250 | none | balance | 8 | " |
| | | | | | |
| B-7 | none | 5% | balance | none | Control, use data from A-7 |
| B-8 | 50 ppm | 5% | balance | 8 | Determine $CO_2$ effect on NOx LC50 |
| B-9 | 100 ppm | 5% | balance | 8 | " |
| B-10 | 150 ppm | 5% | balance | 8 | " |
| B-11 | 200 ppm | 5% | balance | 8 | " |
| B-12 | 250 ppm | 5% | balance | 8 | " |

LC50 => "Lethal Concentration (to) 50% of animals"

The animals will be exposed to levels of test gases at concentration levels sufficient to cause lethality. Since the toxicological endpoint of the N-gas model in its current form is death (of 50% of the exposed animals), sufficient exposures at, above and below the predicted $LC_{50}$ concentration will be required to accurately determine the actual $LC_{50}$ values. The effects of the test gases singly and in combination will be noted and the resulting $LC_{50}$ values will be incorporated into the N-gas model.

Based on the experiments done at NIST to add HCN to the N-gas model (Levin, et al, 1986), approximately six exposure concentrations were required to bracket the $LC_{50}$ value for HCN in air. Most exposure concentrations were done in duplicate (i.e. repeated with a second group of six rats). An additional six to eight exposure concentrations were required to define the empirical relationship/effects of $CO_2$/CO on the air/HCN mix. Since there is strong evidence of non-additive effects between $NO_2$ and other gases, (Gelzleichter, et al, 1992), it is expected that additional exposure combinations will have to be evaluated to bracket $LC_{50}$ values for the expanded N-gas model. See Table II for a matrix of the proposed individual experiments. Eight rats will be used in each experiment: six for the exposure/observation and two for blood collection & sacrifice. Animal handling procedures and sampling procedures are detailed in Section B of this protocol. Detailed descriptions of the exposure chamber, gas delivery methods and sampling techniques are contained in Sections C and D.

4. Validation of the expanded N-gas model. Animal testing will be conducted as described in paragraph D. to validate the empirical constants and predictive value of the expanded N-gas model (for $LC_{50}$ values). Table III is a matrix of proposed experiments to be conducted for the validation phase of this protocol. As previously noted, the concentrations and combinations listed in Table III are subject to refinement based on the

## TABLE III

### Verification of Expanded N-gas Model

| Run # | $NO_2$conc, ppm | CO conc, ppm | $CO_2$ conc, % | Air, breathing | Number of rats | Objective |
|---|---|---|---|---|---|---|
| | | | | | | |
| C-1 | none | none | none | balance | none | Control, Use data from A-1 |
| C-2 | 200 ppm | 2500 ppm | none | balance | 8 | Verify additive effect of NOx & CO |
| C-3 | 100 ppm | 2500 ppm | 5% | balance | 8 | Verify $CO_2$ effects |
| C-4 | 50 ppm | 4000 ppm | none | balance | 8 | Verify additive effect of NOx & CO |
| C-5 | 50 ppm | 4000 ppm | 5% | balance | 8 | Verify $CO_2$ effects |
| C-6 | 150 ppm | 3000 ppm | none | balance | 8 | Verify model at sublethal conc |

results of phases 2 & 3.

B. Animals

Although the current N-gas model was developed using male Fischer 344 rats, the Sprague-Dawley rat is proposed for this protocol. In contrast to the Fischer 344 (an inbred rat), the Sprague-Dawley is an outbred rat and is more commonly used in acute exposure studies. If statistically significant differences are found in the $LC_{50}$ values obtained during the first phase of animal testing (the $CO/CO_2$ effects study), a decision will be made to either continue with the Sprague-Dawley or to revert to use of the Fischer 344 strain.

Male rats weighing 250 to 350 grams will be used in these exposures. Upon arrival, the rats will be quarantined in accordance with WRAIR Division of Veterinary Medicine procedures. The animals will be housed no more than 2 rats to a cage and will be in a separate rodent room. Room temperature will be maintained at 18 to 26 degrees C and relative humidity at 40 - 70 %; light will be regulated to provide 12-hour photoperiods. The animals will be provided food and water *ad libitum*. The animals will be fasted overnight prior to exposures (Sanders, et al, 1993).

As blood chemistry and lethality data are to be collected on the animals during and after exposure, there is no drug therapy planned for relief of pain. Details of blood collection techniques are in Section D & Appendix B. No animal will be exposed more than once to any combination of toxic gases. At the conclusion of the 14 day exposure period, surviving animals will be delivered to WRAIR Division of Medicine Investigator, Dr. N. M. Elsayed, for his studies of oxidative stress in rats. The animals will be anesthesized with sodium pentobarbital (60 mg/kg body weight), IP or IM, the chest cavity will be opened and the animal euthansized by exsaungination. Biochemical analysis of blood and lung tissue will be performed as described in Protocol # MO5-92 (Ref 3). Any surviving

animals which cannot be utilized by Dr. Elsayed will be euthanized by $CO_2$ exposure chamber.

C. Animal Exposure System & Chemical Analysis

A modified 200 liter rectangular Plexiglas chamber designed for the NBS Toxicity Test Method (Levin, et al, 1982) will be used in both the non-animal and animal exposures. For these experiments, the test gases will not be generated by combustion, but instead, by controlled delivery into the chamber of reagent grade pure gas (or gas mixtures in air) from cylinders located external to the chamber. The entire exposure facility, including delivery cylinders, will be located under a fume hood. Air in the lab will be monitored during exposure studies to ensure that ambient levels of toxic gases do not exceed OSHA limits. Chamber gases will be sampled and concentrations recorded at least every 60 seconds. Carbon monoxide and carbon dioxide concentrations will be measured by nondispersive infrared spectroscopy, oxygen concentrations will be measured by a paramagnetic analyzer. Nitrogen dioxide and nitric oxide levels will be monitored by a chemiluminescent NOx analyzer.

D. Test Procedure

Eight rats will be exposed in each experiment. Two of the animals will be selected at random for blood collection/sacrifice. Each animal will be marked with color coded stripes on the tail designating the exposure gases and given a number from 1 to 8. These numbers will be used for recording weights and other data. The animals will be placed in restrainers which will then be inserted into portholes located along the front of the exposure chamber such that only the noses are exposed. The portholes are fitted with rubber stoppers. After the exposure chamber has been charged and the atmosphere has equilibrated, the restrainers will be fully inserted and the stoppers will fall into the chamber, and the animal exposure begins.

Animals will be monitored throughout the exposure period and the time of death noted. The two rats identified for blood collection will be sampled alternately at 0 minutes, 5 minutes, 15 minutes, 30 minutes and one hour post exposure. Blood samples will be taken by tail artery stick using a 27 gauge needle while the animals remain in the exposure chamber and in the restrainers. No more than 0.8 mls of blood will be taken at any one sample period nor will any animal be sampled more than three times. If simple puncture of the caudal artery does not yield adequate quantities of blood, then the tail artery catheterization procedure detailed in Appendix B will be utilized on future exposures. Samples will be analyzed for hemoglobin, methemoglobin, carboxyhemoglobin, blood gases and pH. At the conclusion of the sampling period, the (two) animals will be euthanized by $CO_2$ exposure or by cervical dislocation.

At the conclusion of the exposure time, the restrainers will be removed and the portholes immediately resealed. Any deaths will be verified and noted prior to disposition of the animals. The six rats in the exposure group will be removed from their restrainers. Even immediately after exposure, there is no possibility of the animal respiring any significant level of exposure gas and the animals are safe to handle. The (remaining) animals will be returned to their cages and monitored for 14 days. Monitoring will be conducted twice daily (at least once daily on holidays & weekends) by either the PI (S. Smith, work 301-295-2755 or home 301-208-8027) or by a designated technician. Weight will be recorded daily, as will observations about the general condition of the animals. Changes in sniffing behavior, listlessness, aggression, changes in breathing patterns and appearance will all be noted. The day of death will be noted for each animal, if applicable. Carcasses shall be tagged and bagged and placed in the freezer.

E. Data Analysis

Based on individual gas concentrations, exposure times and the equivalent concentration (defined as the product of time of exposure and toxicant concentration), the $LC_{50}$ values for $NO_2$ singly and in combination with varied levels of carbon monoxide and carbon

dioxide will be determined.

Blood parameters and respiratory data will be plotted against time and concentration. Effective doses will also be calculated as a function of minute volume versus concentration in an effort to determine effects of increased respiration rates [due to $CO_2$] on the apparent $LC_{50}$ values obtained for CO and $NO_2$.

$LC_{50}$ values and the 95% confidence limits will be calculated by the statistical method of Litchfield and Wilcoxon (Litchfield, et al, 1949). Determinations of any true synergistic or additive effects of $NO_2$ in combination with other gases will be made by performing two-way ANOVA analysis (Gelzleichter, et al, 1992). The N-gas model will be expanded/refined as necessary to reflect these findings and will be verified by animal tests as described in Paragraph D. of this Section.

## APPENDIX A - REFERENCES


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## APPENDIX B

PROCEDURE FOR CATHERIZATION OF TAIL ARTERY

The procedure takes about 10 -15 minutes and will be done about 24 hours prior to toxic gas exposure. Rat is anesthetized with Ketamine (4 to 8 mg/100 gm) & xylazine, tradename Rompun (1 mg/100 gm) administered IM, site to be the large muscle mass of the rear leg. If required,additional doses of ketamine may be administered intro-operatively. The animal will be placed in dorsal recumbancy on a secure surface. The surgical site will be aseptically prepared and aseptic technique will be used in accordance with WRAIR policy letter SGRD-UWN # 93-28, "Survival Surgery & Recovery for Rodents". An incision is made along the centerline of the tail, about 1/8 inch deep, about 1 1/2 inches long and sufficient to reveal the tail artery. Connective tissue is removed for a length of approximately 3/4 inch along the artery. The section of artery is lifted out, using forceps, and sutured distally. The area is irrigated with 1% xylocaine. A 23-gauge needle is used to puncture a hole in the top of the artery. Capillary tubing, PE-50, ID 0.023"/OD 0.038", is inserted into the artery at least 1 1/2 inches. The catheter will be flushed every eight hours (after placement) and between each sampling with 0.1 ml of 0.1% Heparin/saline to prevent clotting. At the time of exposure, the end of the tubing is fitted with a BD-23 gauge tubing adaptor and a syringe with heparin/saline solution. The tubing is secured in place with 00-suture above and below the tubing entry point. Then the tail incision is closed with two or three sutures. Surgical grade cyanoacrylate adhesive will be used as needed to seal the skin surface. After placement of the catheter, an antibiotic ointment (Bacitracin w/o steroid) shall be applied to the site and a bandage applied to prevent infection. Samples are taken as needed (up to a maximum of 3 samples in 3 hours), with the blood volume taken (per sampling) not to exceed 0.8 ml using a tuberculin syringe with lithium heparin. At the conclusion of the procedure, the animal will be euthanized.

## APPENDIX C

Justification for "P" Rating Under USDA Code

In order to validate "N-gas model" (an empirical bioanalytical tool to minimize the number of animals required to predict the lethality of mixtures of toxic combustion gases), minimal quantities of animals are exposed to potentially lethal levels of combinations of those gases under strictly controlled conditions. Drug intervention could lead to unrealistic survival rates, rendering the test results inaccurate and the resulting empirical tool non-predictive.